Journal of Experimental Marine Biology and Ecology 511 (2019) 68–74

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Variation in metabolic rate and a test of differential sensitivity to temperature in populations of woolly sculpin (*Clinocottus analis*)

Racine E. Rangel*, Darren W. Johnson

Department of Biological Sciences, California State University Long Beach, 1250 Bellflower Blvd, Long Beach, CA 90840, USA

ARTICLE INFO

Keywords:
Biogeography
Climate change
Individual variation
Metabolism
Oxygen consumption
Thermal sensitivity

ABSTRACT

Variation in water temperature can have important effects on the energetics underlying metabolism, growth, and reproduction of many organisms. In order to anticipate how a species' allocation of energy may change in a warming ocean, we need to know the degree to which processes such as metabolic rate change with temperature (*i.e.,* the thermal sensitivity of metabolism, Q_{10}). In the context of long-term climate change, it will also be helpful to know whether local populations that experience different thermal regimes exhibit differences in thermal sensitivity. If so, it would indicate that thermal sensitivities of populations may adjust *via* acclimation or adaptation. We measured metabolic rates of Woolly Sculpin collected from two distant populations (Santa Cruz (SC) and Los Angeles (LA) counties, California, USA) and housed in a common environment. Using respirometry, we estimated resting mass-specific oxygen consumption (MO_2) and calculated metabolic rates (MR) at temperatures of 12 °C, 16 °C, and 20 °C for 59 fish. The Q_{10} value across this range of temperatures was 2.92. Populations did not differ with respect to overall MO_2, nor did they vary in their degree of thermal sensitivity. However, the LA population had greater variation in MO_2 among individuals when compared to the SC population. Larger variation in overall MO_2 among individuals within specific populations may reflect greater genetic diversity, and this may buffer some populations against the effect of future increases in water temperature.

1. Introduction

Many marine organisms exist over large geographic ranges. In part, this is because most marine species have a planktonic stage that is capable of dispersing with ocean currents, and even species whose adults move very little (*e.g.,* many algae, benthic invertebrates, and reef fishes) are frequently distributed across ranges that span from hundreds to many thousands of kilometers (Lessios et al., 1998; reviewed by Shanks, 2009; Shanks et al., 2003). Over these ranges local populations often differ in their exposure to environmental conditions such as pH, nutrient availability, salinity, and oxygen levels (Bustamante et al., 1995; Feely et al., 2008; reviewed by Sanford and Kelly, 2011). Within coastal habitats, populations may also experience very different water temperatures (Legaard and Thomas, 2006). Regional variations in both average temperature and the range of temperatures that populations experience can be extremely important for marine species because temperature influences physiological processes such as enzymatic activity, maximum oxygen uptake, metabolic rate, and growth rate (Angilletta, 2009; Clarke and Johnston, 1999; Hazel and Prosser, 1974; Somero, 2005).

For species that reside over large geographic ranges, local populations may respond differently to environmental gradients, and some populations may exhibit consistent differences in their physiological responses to temperature change. The degree to which a physiological rate changes with temperature (hereafter referred to as thermal sensitivity) is a property that may vary among individuals and populations, and a property that is important for the bioenergetics of organisms in a warming ocean. For example, specific populations of the marine intertidal goby, *Gillichthys mirabilis,* that resided in warmer and more variable temperature conditions had a lower thermal sensitivity of oxygen consumption and were able to cope with changes in temperature better than their northern counterparts that experienced a more thermally stable environment (Barlow, 1961). Having a lower sensitivity to temperature change may allow certain populations to persist under changing environmental conditions. For example, thermally sensitive populations of various species have been observed to undergo range contractions, increased parasitism, and even local extinctions due to increasing water temperature within their regional environments (Harley et al., 2006; Poulin and Mouritsen, 2006; Sorte et al., 2011). However, a one-time measurement of thermal sensitivity may not always indicate the potential for populations to cope with changing temperature. Some populations may be able to thermally acclimate and

* Corresponding author.
E-mail addresses: raciner@uci.edu (R.E. Rangel), darren.johnson@csulb.edu (D.W. Johnson).

https://doi.org/10.1016/j.jembe.2018.11.007
Received 8 June 2018; Received in revised form 18 November 2018; Accepted 19 November 2018
Available online 27 November 2018
0022-0981/ © 2018 Published by Elsevier B.V.

in some cases permanently modify their physiological responses. These modifications may enable them to persist under the effects of temperature increase.

One way in which populations may acclimate is through developmental canalization where an individual's physiological response to the thermal gradient they encounter throughout life is determined by the thermal conditions they experienced during early ontogeny (Angilletta, 2009). Thermal sensitivities could then be unique to specific local populations that developed in different thermal environments. For example, of four different populations of intertidal snails (*Littorina spp.)* along the northwest Atlantic Ocean, the highest latitudinal or cold-acclimated population had a reduced acclimation capacity to increasing temperature when compared to its southern, warm-acclimated counterparts (Sorte et al., 2011). Depending on thermal history local populations may have very different acclimation capacities and may vary in their response to further temperature increase (Donelson and Munday, 2012; Stillman, 2003).

Another mechanism that can lead to regional differences in thermal sensitivities is local adaptation. This occurs when local genotypes provide a greater advantage under local conditions than do foreign genotypes (Kawecki and Ebert, 2004; Slatkin, 1987). For example, F_2 populations of a marine intertidal whelk, *Nucella canaliculata,* collected from central Oregon had significantly higher upper lethal temperatures compared to conspecifics from Central California (Kuo and Sanford, 2009). These authors concluded that whelks from Central California are potentially less thermally tolerant because they came from sites that experience lower variation in heat stress during seasonal and daily tide cycles. However, if dispersal is high among populations, as it may be for many marine species, then gene flow may counteract selection of local traits and local adaptation will be unlikely to persist (Grosberg and Cunningham, 2001; Lenormand, 2002). Therefore, the life history and the degree of larval dispersal are important in determining whether or not certain populations have the ability to locally adapt to their thermal environment (Sanford and Kelly, 2011; Warner and Cowen, 2002).

Testing for variation in thermal sensitivity between populations can be accomplished by measuring metabolic thermal reaction norms, which help to describe the relationship between metabolic rates and changing temperature (Angilletta Jr et al., 2003; Careau et al., 2014). Metabolic thermal reaction norms may be envisioned as functions that can vary in elevation and/or slope, and both of these attributes may vary across different levels of biological organization (*e.g.,* among-individuals and/or between populations). Baseline metabolic rates have been shown to vary among individuals of the same population, and sometimes the baseline metabolic rates may be correlated with the thermal sensitivities of individuals (Norin and Malte, 2012; Norin et al., 2016). For example, Careau et al. (2014) showed that for wild-caught slimy salamanders (*Plethodon albagula*) tested at four different temperatures, individuals with high or low metabolic rates overall either exhibited low or high thermal sensitivity, respectively, when undergoing changes in temperature. Norin et al. (2016) found similar results in juvenile barramundi (*Lates calcarifer*) fish, where individuals that were observed to have lower metabolic rates under acclimated conditions had high metabolic rates when exposed to acute temperature change, however individuals with high metabolic rates under acclimated conditions showed little change when exposed to acute temperature. Therefore, populations that differ in their thermal gradients may respond very differently to temperature increase depending on the amount of among-individual variation that is within the population.

In this study we evaluated differences in thermal sensitivities for two populations of woolly sculpin (*Clinocottus analis*), a common intertidal fish found over a broad geographic range from Cape Mendocino in Northern California, to central Baja California, Mexico (Love, 2011). Populations of *C. analis* may have limited potential for long-distance dispersal of larvae because adults spawn in the intertidal and larvae have been found very close to shore and adjacent to adult habitat (Waples, 1987). Analyses of allozymes and comparisons of gene frequencies among populations that are separated by 173-608 km suggest that rates of gene flow may be moderate at these scales (Waples, 1987; also see Yoshiyama and Sassaman, 1987). Populations that are separated by long distances (*e.g.,* many hundreds of km) may experience lower gene flow, especially if oceanographic features such as major currents restrict larval dispersal (Cetina-Heredia et al., 2015; Han et al., 2016; Wright et al., 2015). It is possible that sculpin from distant populations that experience different thermal regimes may have different sensitivities to changes in water temperature. Thus the aims of this study were to determine whether two widely-separated populations differed with respect to both resting mass-specific metabolic rates, and thermal sensitivity. We expected that *C. analis* from the more southern populations (LA) would have metabolic rates that are less sensitive to temperature change because the LA population is more likely to experience greater fluctuations in thermal conditions on a daily basis when compared to their northern counterparts (SC).

2. Methods

2.1. Study regions and temperature variation

We studied populations of *C. analis* at two locations that are separated by 615 km and differ in respect to the temperature ranges they experience (Appendix 1). Davenport Landing is located within Central California in Santa Cruz County (SC), and is characterized by cooler temperatures that vary little throughout the year. For example, average water temperature throughout 2016 was 13.9 with a monthly standard deviation of 1.04 °C (buoy station 46,092 of the National Data Buoy Center National Oceanographic and Atmospheric Administration; http://www.ndbc.noaa.gov/). Palos Verdes is located in Southern California within Los Angeles County (LA), and is characterized by warmer temperatures and a higher degree of variability (Appendix 1). For example, Palos Verdes average water temperature was 17.8 with a monthly standard deviation of 1.21 °C for 2016 (station 46,256, http://www.ndbc.noaa.gov/). Along with this difference in average sea surface temperature, the temperature ranges that *C. analis* likely experience at these locations differ substantially. For example, Leong et al. (2017) found that tide pools locations in Santa Cruz County over the summer months (June – August) had an average daily water temperature range of approximately 4.6 °C whereas tide pools in San Diego and Santa Catalina Island (locations relatively close to our study population in LA county) had an average daily water temperature range of 10.0 °C. Thus, *C. analis* that inhabit different parts of the California coastline may be exposed to very differing thermal gradients on both a daily and yearly basis.

2.2. Fish collection and acclimation

A total of 60 individuals were collected by dipnet at the two study sites (LA = 26, SC = 34). At time of collections tide pools in SC ranged from 11 to 13 °C, and in LA tide pools ranged from 18 to 22 °C. Fish were then transported in an insulated cooler filled with aerated seawater that was occasionally replenished with seawater from the collection location. In < 8 h from collection fish were delivered to the California State University Long Beach (CSULB) Marine Laboratory. Upon arrival to CSULB fish were placed in fresh seawater collected in carboys at each collection site and this water was mixed with clean 16 °C or 24 °C marine lab seawater to match the water temperature that the individuals were transported in. Fish were then slowly acclimated to 16 °C over a 24 h period by being placed in a temperature controlled cold room. Individuals underwent a quarantine period that lasted for an additional 24-h period before being placed in 10 (27-l) tanks that were attached to a 3700-l re-circulating saltwater tank system with a 12-h light: 12-h dark photoperiod. Fish were separated based on population and there were 6 individuals per holding tank throughout the duration of the study. Water quality parameters (including temperature, DO,

salinity, ammonia, nitrite, nitrate, and pH) were measured daily in the holding tanks, and nitrogenous waste was low throughout the study. *Clinocottus analis* specimens were blotted dry and weighed to the nearest 0.01 g on a digital scale. Individuals were measured to obtain standard length (SL), and marked with a small subcutaneous injection of colored elastomer (Northwest Marine Technologies, Shaw Island, WA), using a 28-gauge needle on the ventral side of the fish just adjacent to the anterior portion of the anal fin. A combination of tank, tag location, and color was used to give each individual a unique identifier. Previous studies have determined no significant effect of this tagging procedure on mortality and growth of various sculpin species of similar sizes and smaller (Pfister, 1996, 1997, 2006; Ritter, 2008, 2009; Ritter and Preisler, 2006), and we noticed no effects of tagging on behavior. Fish were held at 16 °C, and a salinity of 34 ppt for three weeks before the start of experiments to minimize any effects of transportation and handling. Change to subsequent temperature treatments occurred with a 1 °C change every other day until desired temperatures of 12 °C was reached. Individuals were then allowed to acclimate for two weeks prior to testing to minimize the effects of short-term metabolic compensation (Barrionuevo and Fernandes, 1998). Once fish were tested at 12 °C, they we re-acclimated to 16 °C and tested, then acclimated to 20 °C and tested. Most fish were tested at all three temperatures, but some individuals were excluded from analysis if the individual moved excessively throughout the trial and a few sculpin died before testing at all temperatures could occur. Of the 26 sculpin collected at LA there were 21 tested at 12 °C, 26 at 16 °C, and 14 tested at 20 °C. Of the 34 sculpin collected at SC there were 32 tested at 12 °C, 34 tested at 16 °C, and 19 tested at 20 °C. The weights of sculpin varied somewhat between source populations and among temperature groupings, but the effects of variation in mass were accounted for by examining mass-specific oxygen consumption rates (see *Measuring oxygen consumption and metabolic rate* section). Mean (SD) values of weights for at LA for the 12 °C, 16 °C, and 20 °C treatments were 17.73 (12.38), 15.67 (8.41), 17.56 (8.56) g, and the SC population weights were 15.10 (7.22), 13.42 (5.75), and 12.48 (4.12) g. Fish were fed a mixed diet of frozen mysid shrimp, brine shrimp, and squid pieces from San Francisco Bay Brand *ad libitum* once a day.

2.3. Measuring oxygen consumption and metabolic rate

To measure resting metabolic rates, we recorded oxygen consumption using an optical dissolved oxygen probe within an intermittent-flow respirometry system (Q-box AQUA, Qubit systems, Canada). Sculpin were fasted for > 24-h to minimize metabolic demands due to specific dynamic action (review by Clark et al., 2013). Prior to the start of each set of measurements, individuals were weighed to the nearest 0.01 g, and then placed in an acrylic chamber 12–16 h before each trial to allow for adequate acclimation and minimization of stress (Barlow, 1961). Fish were placed either in a 220 ml chamber (ID: 3.84 cm, L: 15.3 cm) if the standard length of the fish was < 10 cm or a 730 ml chamber (ID: 8.2 cm, L: 16.7 cm) if the standard length of a fish was > 10 cm. Chambers were submerged in a 95-l water bath that was held constant at the desired water temperature treatment and then covered to prevent disturbance (Fig. 1). Continuous flow was maintained at a rate of 757.5 ml min^{-1}. For individuals < 10 cm, water flow occurred for 5 min followed by a 6-min closed circuit period for a total of nine cycles. For individuals > 10 cm, water flow occurred for 10 min, followed by a 20-min closed circuit period, repeated six times. Each closed circuit period was timed to ensure that oxygen within the chamber did not fall below ≈80% of the starting oxygen concentration (see recommednations by Clark et al., 2013). O_2 concentration (mg l^{-1}) within each chamber was recorded every second. A linear regression of oxygen concentration over time (excluding the first minute after each closure of the system) was used to determine the rate at which each fish consumed oxygen, with oxygen consumption rate calculated from the slope of the regression line and then converted into hourly rates. The first slope was excluded from analysis to minimize the residual effects of handling and stress, and then VO_2 ($mgO_2\ L^{-1}\ h^{-1}$) was calculated from the average of the remaining slopes of O_2 consumption (all slopes used in analysis had an r-squared value > 0.90). After each trial, a control trial containing only seawater (a blank) was run under the same protocol as mentioned above in order to account for oxygen consumption by microbes in the seawater. Mass-specific oxygen consumption rates (in units of $mgO_2\ g^{-1}\ h^{-1}$) were calculated using the formula:

$$MO_2 = \frac{R\ x\ (V_{total} - V_{fish}) - (Bl\ x\ V_{total})}{m} \quad (1)$$

where R is the average rate of oxygen consumption for each individual fish ($mgO_2\ L^{-1}\ h^{-1}$), Bl is the rate of oxygen consumption measured for the corresponding seawater blank ($mgO_2\ L^{-1}\ h^{-1}$), V_{total} is the volume of water in the chamber and associated tubing in liters (Fig. 1), V_{fish} is the volume of water (L) displaced by each fish, and m is the mass of the fish (g). After metabolic measurements were completed, displacement volumes were calculated by placing each fish in a graduated cylinder filled with seawater and noting the change in volume. Because oxygen consumption increases with body mass and the relationship is a multiplicative one, dividing oxygen consumption by mass corrects for any differences in mass between groups of fish and allowed for a more direct comparison of the effects of population and temperature on metabolic rates.

2.4. Data analysis

One method of measuring thermal sensitivity is by calculating a Q_{10} coefficient. This is the factor by which a rate changes over a 10 °C change in temperature (Schmidt-Nielsen, 1997). In general, Q_{10} values may be calculated by comparing measured values of a rate (*e.g.* MO_2) at two different temperature values (T_1 and T_2):

$$Q_{10} = \left(\frac{MO_{2T2}}{MO_{2T1}}\right)^{\frac{10}{T2-T1}} \quad (2)$$

However, if rates such as MO_2 are measured at more than two temperatures, we can fit the Q_{10} relationship as a non-linear function that describes the increase in a rate across a broader range of temperature values. In this way, we can get a more precise estimate of Q_{10} by fitting a single model to the entire data sample, rather than calculating Q_{10} values in a piecewise fashion by comparing two temperatures at a time. If we use 0 °C as our baseline temperature (T_1 in Eq. (2)), we can arrange Eq. (2) to describe observed values of MO_2 at a given temperature (T).

$$MO_{2_T} = MO_{2_0}\left(Q_{10}^{\frac{T}{10}}\right) \quad (3)$$

By taking the logarithm of Eq. (3), we may express the logarithm of mass-specific oxygen consumption as a linear function of temperature:

$$\mathrm{Log}(MO_{2_T}) = Log\,(MO_{20}) + Log\,(Q_{10}) \times \left(\frac{T}{10}\right) \quad (4)$$

where MO_{2_0} is a parameter that describes metabolic rate as temperatures approach zero. When the Q_{10} relationship is expressed in this way, a regression of the log of MO_2 on temperature can be used to estimate the parameters of Eq. (4). Specifically, the intercept of the best-fit line can be used to estimate $\log(MO_2)$, and the slope can be used to estimate $\log(Q_{10})$.

We used Eq. (4) as a general basis for describing variation in our measurements of MO_2. One goal of this analysis was to test whether Q_{10} values (our measure of thermal sensitivities) differed among populations. Another goal was to examine among-individual variation in thermal sensitivities. We began with a model with terms for population (*i.e.*, LA *vs.* SC), temperature, and their interaction. These were treated

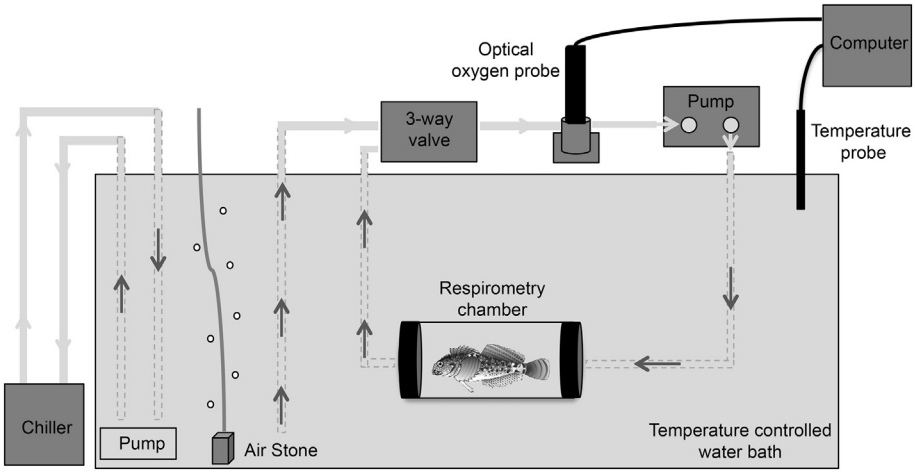

Fig. 1. Schematic representation of intermittent flow-through respirometry system used to measure oxygen consumption rates (VO_2) of woolly sculpin (*Clinocottus analis*). Arrows indicate the direction of water flow. A 3-way valve was used to control whether the system was a closed circuit, or flushed with fresh seawater.

as fixed effects and were our main variables of interest. For a two-population comparison, the fixed effects of the model can be expressed as

$$\mathrm{Log}(MO_{2T}) = (\propto_1 + \propto_2 P_2) + (\beta_1 + \beta_2 P_2) \times \left(\frac{T}{10}\right) \quad (5)$$

where $P_2 = 0$ if the fish came from the reference population, or 1 if the fish came from the other population. The test of a main, population effect is a test of whether average values of log (MO_2) values differed between populations and whether α_2 is significantly different from zero. The value of $\log(MO_{2_0})$ in the reference population is α_1 and the value of $\log(MO_{2_0})$ in the other population is $\alpha_1 + \alpha_2$. The test of a main effect of temperature is a test of whether β_1 is significantly different from zero. The test of the interaction term is a test of whether Q_{10} values differed among populations and whether β_2 significantly different from zero. The value Q_{10} in the reference population β_1 and the value of log Q_{10} in the other population is $\beta_1 + \beta_2$.

We also included random effects to account for potential similarities in MO_2 measurements made on fish inhabiting the same tanks and for potential similarities of fish measured multiple times, but at different temperatures. These can be included as additional terms on the right hand side of Eq. (5), but for the sake of simplicity, we did not list these because we evaluated several different combinations of random effects and used a model selection procedure to choose the appropriate set of random effects (see below). The grouping structure for our random effects was individual fish nested within tanks. Each of these random effects could have affected differences in MO_2 rates overall, thermal sensitivity (Q_{10} values), or both. To accommodate these possibilities, we compared the relative fit of three different models, all of which had the same fixed effects structure (population, temperature, and their interaction), but different structures for the random effects. The simplest model allowed for the intercept, $\log(MO_{2_0})$, to vary among individuals and tanks. This was coded as (1|Tank/Ind) using the lmer package in R (Bates et al., 2014). The next model allowed both the intercept and slope to vary in an uncorrelated fashion among tanks and individuals, and was coded as (Temp||Tank/Ind). The third model allowed both the intercept and slope to vary in a correlated fashion and was coded (Temp|Tank/Ind). In our analyses, we first compared random effects structures by using likelihood ratio tests to evaluate whether the more complex models were significantly better at explaining the variation in the data. Unless the more complex models were justified by a substantial improvement in model fit, we used the simpler version of the random effects structure in subsequent analyses. Once the appropriate set of random effects was selected, we used Wald's tests to test the significance of fixed factors and to evaluate among-population variation in both Q_{10} and overall MO_2 values.

A second focus of this study was to evaluate whether the level of among-individual variation differed between our study populations. Our main analyses indicated no effect of population or population x temperature (see *Results* section). We therefore fit site-specific models to the data in order to isolate and compare the random effect variation (*i.e.,* among-individual) for the two populations. Because the overall analyses indicated that individual variation was best summarized by random effects where the intercepts ($\log(MO_{2_0})$ values) were free to vary among tanks and individuals. For each population we calculated the among-individual component of variation as a proportion of the total variation in ($\log(MO_{2_0})$). Total variation corrected for the effects of temperature and was calculated by summing the among-individual, among-tank, and within-individual (residual) variance components estimated by our random effects model. We then compared proportional levels of among-individual variation for the two populations.

3. Results

3.1. Selecting the random effects structure

The model with the most complex random effects structure (slopes, intercepts, and their correlation were random effects that could vary among tanks and individuals) produced only a marginal increase in likelihood and despite the additional flexibility, did not provide a significant improvement in model fit when compared to the simplest model in which intercepts were allowed to vary among tank and individual as random effects. The log likelihood of the complex model was −105.9 whereas the log likelihood for the reduced model was −107.2. A Likelihood Ratio Test indicated a non-significant improvement in model fit (Drop in deviance = 2.577, df = 4, $P = .631$). Similarly, the model with intermediate complexity (slopes and intercepts were included as uncorrelated random effects that could vary with tank and individual) did not improve likelihood of model fit. In fact, this model converged on version where virtually all of the random effect variation was in the intercepts, and there was near-zero variance in the slopes. Even though the intermediate model was more flexible, when fit to data, it converged on our simplest model, in which only intercepts were allowed to vary. The log likelihood values were identical to two decimal places (both were − 107.2) despite the more complex model having 2 fewer degrees of freedom (Drop in deviance = 0.00, df = 2, $P = 1.00$). In subsequent analyses, we proceeded with a random effects structure in which intercepts were included as random effects that varied among tanks and individual sculpin.

Table 1
Estimates of thermal sensitivities of MO_2 (Q_{10} values) and baseline MO_2 rates for each of our study populations. For further details of parameters interpretation and estimation, see Eqs. (3) and (5) in the main text.

Population	Parameter	Estimate	95% CI	
			LCL	UCL
LA	MO_{2_0}	0.0369	0.0185	0.0736
	Q_{10}	2.564	1.659	3.964
SC	MO_{2_0}	0.025	0.0141	0.0442
	Q_{10}	3.19	2.22	4.589
Pooled	MO_{2_0}	0.0292	0.0189	0.0453
	Q_{10}	2.921	2.213	3.856

3.2. Effects of source population on metabolism and thermal sensitivity

There was no evidence that thermal sensitivity varied between the two populations. The interaction term testing whether the effects of temperature depended on source population was not significant ($\beta_2 = 0.219$, SE = 0.284, Wald's test: $P = .440$) and the 95% confidence intervals for Q_{10} values overlapped substantially for the two populations (Table 1). There was a significant, overall increase in MO_2 with increasing temperature, ($\beta_1 = 0.942$, SE = 0.218, Wald's test: $P = 1.517 \times 10–5$), but after accounting for the effects of temperature, there were no consistent differences in average MO_2 values between the two populations ($\alpha_2 = -0.391$, SE = 0.448, Wald's test: $P = .383$) (Table 1, Fig. 2). Because there was no significant difference in MO_2 between the SC and LA populations (*i.e.,* no population effect), and because the effect of temperature did not depend on population (*i.e.,* no interaction effect), we dropped the terms for interaction and population and calculated an overall Q_{10} for *C. analis* from both sites. In this analysis, the temperature coefficient estimates the natural log of Q_{10} (see Eq. (4)), and we calculated *C. analis* to have a Q_{10} of 2.92 (95% CI: 2.21–3.86) across the experimental temperatures (12 °C – 20 °C) suggesting that for a 10 °C change in temperature metabolic rate would increase by a factor of 2.92.

3.3. Individual variation of metabolism within populations

Between the two populations there was a differing amount of individual variation in MO_2 across all temperatures (Fig. 2). The LA population had a higher amount of consistent, among-individual variation such that individuals that had a higher metabolic rate at one temperature tended to have high metabolic rates at the other temperatures as well. This among-individual component of variation accounted for 35.2% of the variation. In contrast, our analysis estimated little-to-no consistent, among-individual variation in the SC population (Fig. 3). In this population, the among-individual component was estimated to account for < 1% of the total variation.

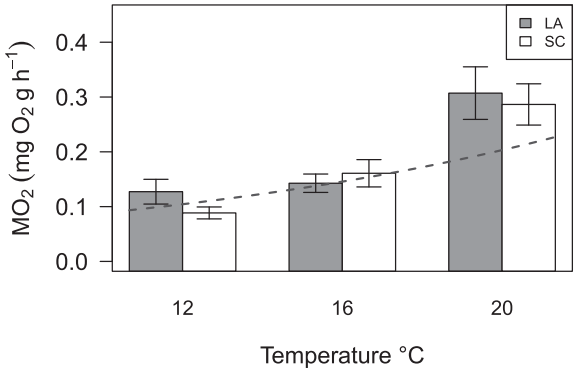

Fig. 2. Effects of temperature on mass-specific oxygen consumption (MO_2) of *C. analis*. Data presented are means ± SE for fish at tested temperatures from two locations. Gray bars indicate data from Los Angeles (LA) and white bars are from Santa Cruz (SC). Line represents predictions from a continuous, temperature-dependent model of mass-specific oxygen consumption (see Eq. (4) in the main text).

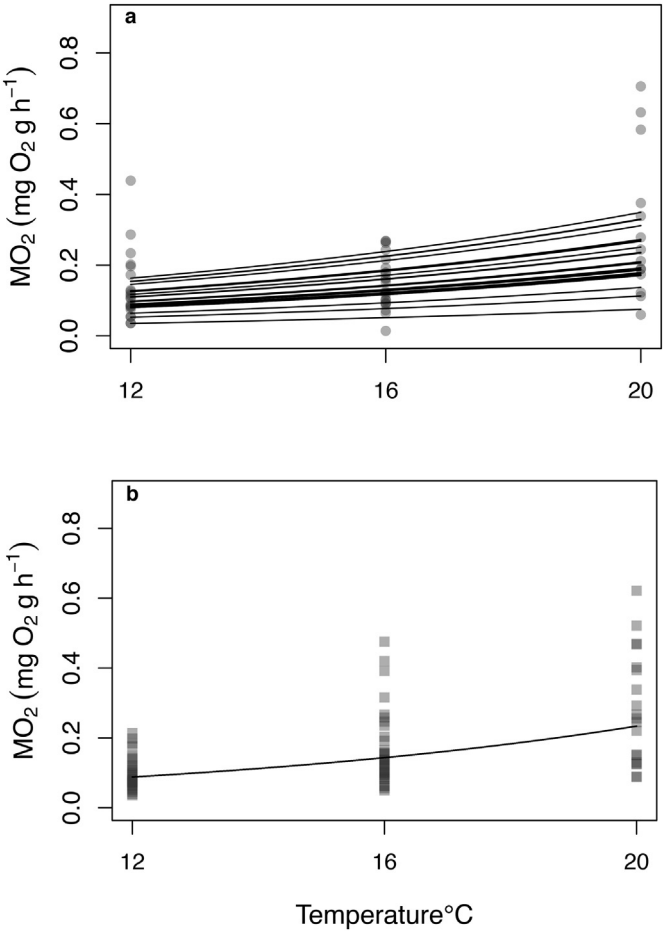

Fig. 3. Individual variation in mass-specific oxygen consumption (MO_2) between two populations. Panel (a) displays data for fish from the Los Angeles (LA) populations (circles) across all temperature treatments (b) displays data for fish from Santa Cruz (SC) populations (squares) across all temperature treatments. Lines indicate model predictions for each individual's average MO_2 values across the range of temperatures, LA has a total of 24 lines and SC has a total of 36 lines though all of these fall approximately on the same line.

4. Discussion

This study revealed important information about the thermal sensitivity and metabolic rate of *C. analis* populations from varying geographic locations. In particular, we found no difference between populations in their overall sensitivity to temperature change. These results were unexpected, as previous studies have shown other species that inhabit large geographic areas and experience different thermal gradients tend to exhibit differences in their physiological responses to temperature change. For example, geographically separated populations of adult sockeye salmon (*Oncorhynchus nerka*), that experienced the coldest water exhibited the lowest optimal temperature for aerobic scope (*i.e.,* the difference between resting and maximal oxygen consumption rates), and populations from warmer waters had the highest optimal temperatures for aerobic scope (Eliason et al., 2011). A similar trend was also observed in Atlantic populations of the common killifish (*Fundulus heteroclitus)*, where southern populations that experience larger daily thermal gradients had upper lethal thermal limits that were on average 1.5 °C higher than their northern counterparts when tested

at multiple acclimated temperatures (Fangue et al., 2006). However, our study observed no differences between the Los Angeles and Santa Cruz population's thermal sensitivities, even though the southern (LA) population tends to experience much greater temperature variation within tide pool habitats (Leong et al., 2017).

In addition, in another species of intertidal sculpin that ranges over a large geographic area (the tidepool sculpin, *Oligocottus snyderi*), Morris (1961) found that Southern California populations that inhabit warmer waters had lower Q_{10} values when compared to their more northern counterparts in Oregon, and their overall oxygen consumption rates were significantly different. For *O. snyderi* Morris (1961) calculated oxygen consumption values that ranged from approximately 0.04 to 0.5 $mlO_2\ g^{-1}\ h^{-1}$. Since *C. analis* populations inhabit a similar geographic range as *O. snyderi,* we expected similar geographical differences where the more southern (LA) population would exhibit a lower Q_{10} value as the LA population is exposed to overall warmer water on average than the northern (SC) population (Leong et al., 2017). Guderley and Blier (1988) have also suggested that optimal performance and lowest thermal sensitivity occur within the temperature ranges that the individuals most frequently encounter. *Clinocottus analis* LA and SC populations differ in their thermal ranges (10 °C daily range and 5 °C daily range, respectively). If these two populations had different thermal optima and differences between their thermal sensitivity then we would have been able to detect those differences between the populations throughout our experiments and across our experimental temperature range.

A potential explanation for why we saw no differences between our study populations is that our individuals may have acclimated to laboratory conditions, and lowered their metabolic rates and/or Q_{10} values. Down-regulation of Q_{10} and metabolic rates have taken place in other fish species in captivity (Xie et al., 2017; Zeng et al., 2011). These studies however, were testing individuals from a single location. Other studies that have tested multiple, distantly located populations, similar to our study, have noticed varying physiological responses in populations even after a period of acclimation. For example, Lee et al. (2003) found that differences in metabolic rates of acclimated captive adult salmon from different source populations was largely explained by the individuals' thermal histories. If differences between the LA and SC population existed, down-regulation of metabolism would obfuscate these differences only if down-regulation was stronger for one of the populations. This scenario is possible, but less likely than the scenario where there are no significant differences in MO_2 and Q_{10} populations.

Regarding thermal sensitivity, our results suggest that *C. analis* as a species, has a metabolic rate that is moderately sensitive to temperature change (Q_{10} = 2.92 when all data are considered). This value falls within the range (Q_{10} = 1.06–7.20) observed for fishes of similar size and behavior (Rangel and Johnson, 2018). *Clinocottus analis* does not move long distances and therefore must have some physiological mechanism to cope with temperature changes individuals experience on a daily basis. Our Q_{10} of 2.92 (95% CI: 2.21–3.86) is also very similar to the single value of 2.9 reported by Morris (1961), who studied metabolic rates within one population from Monterey, California. Our study focused on a smaller range of temperatures that *C. analis* experiences regularly rather than testing them at extreme temperatures (*i.e.,* temperatures during low tide events), in which differences in thermal sensitivity may have been observed.

Although there were no consistent differences between study populations in regard to overall thermal sensitivity, we did find that the LA population had a greater amount of among-individual variation when compared to the SC population. In the LA population, most of this variation manifested as consistent differences in overall metabolic rates (*e.g.,* individuals with high metabolic rates tended to have high metabolic rates at all experimental temperatures). This phenomenon is illustrated by the variable curves that were estimated for individual fish in Fig. 3; note that the individual curves for the SC population tightly overlap the overall average. One reason for the greater among-individual variation in the LA population may be the presence of greater genetic variation within this population. Such genetic variation may arise from niche diversification and/or recruitment of larvae from a greater variety of source populations. In the SC population there was a high diversity of intertidal sculpin inhabiting the same pools as *C. analis* (*e.g. Oligocottus snyderi*, *Clinocottus recalvus*, and *Oligocottus maculosus*; R. Rangel personal observation). In contrast, *C. analis* was in greater abundance, and the only sculpin species observed in the LA population. A greater amount of interspecific competition may lead to the SC population occupying a narrower niche when compared to the LA population. Having less interspecific competition may have allowed individuals in the LA population to undergo greater specialization when it comes to use of resources (*e.g.*, food and microhabitats) (*e.g.,* Faria and Almada, 2006; Feary and Clements, 2006; Mayr and Berger, 1992). Such specialization may facilitate genetic diversification (Futuyma and Moreno, 1988; Kassen, 2002; Schluter, 2000) leading to greater variation in characteristics such as metabolic rate.

In addition to potential differences in competition between the two populations, the LA population may potentially be receiving greater gene flow from a greater variety of populations than the SC populations. The LA population does reside in a region where ocean currents are variable (Bograd et al., 2001; Bograd and Lynn, 2003), and is exposed to water flow from both the north and south at different times throughout the year (Hickey et al., 2003; Parrish et al., 1981). Therefore, populations may be regularly receiving recruits from varying regions, thus fostering genetic diversity within this population. A greater in depth study for genetic differences between southern populations of *C. analis* is needed. If among-individual phenotypic variation in metabolic rate has a genetic basis, this may allow for faster rates of adaptation to future changes in water temperatures (Bolnick et al., 2011). Such effects could be particularly important for the LA population, as Southern California recently experienced the most severe El Niño event to date from 2014 to 2016 where water temperatures were sustained at levels 3–5 °C above average temperatures (Zaba and Rudnick, 2016). If the LA population is able to adapt faster, then this population may have a greater chance of surviving future thermal changes.

We found no differences in metabolic rates or thermal sensitivity between populations of *C. analis* over a varying geographic range. Our results from this study do however, suggest that *C. analis* has a relatively low sensitivity to temperature change. As the climate within our oceans continues to change, *C. analis* has the potential to be less affected by warming ocean temperatures (especially compared to other small benthic fishes; Rangel and Johnson, 2018). Being less metabolically sensitive to temperature change may serve as a benefit to *C. analis,* which is a benthic fish that is unable to travel long distances. *Clinocottus analis* will be less likely to undergo costly trade-offs between metabolism and other energy-demanding processes such as growth and reproduction. Among-individual variation in metabolic rates within our study populations may also help to serve as a natural buffer against climactic events such as El Niño events, which are expected to become more frequent and intense as climate warms (Cai et al., 2014; Wang et al., 2017). We suggest that future studies of metabolism and energetics take into account how populations that are distantly located may differ in their levels of among-individual variation in metabolic responses to temperature change. Determining whether other marine fishes that exist over a large geographic range similar to *C. analis,* also differ in their physiological responses to temperature will be important in determining whether populations will be able to thermally adapt or acclimate to future increases in ocean temperatures.

Acknowledgments

We would like to thank Sarah Luongo, David Lizarraga, Amanda Benas, and Kathryn Scafidi for help with collection of specimens, and Connor White and Bengt Allen for use of equipment and data analysis assistance. We would like to thank Larry Allen for supplying the

drawing of *C. analis* and Emily Meese for supplying the map of *C. analis* collecting locations. R. Rangel received support from Southern California Tuna Club, Los Angeles Rod and Reel Club, the Reish Grant for Graduate Research, and Council on Ocean Affairs, Science and Technology graduate student research award.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2018.11.007.

References

Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press.
Angilletta Jr., M.J., Wilson, R.S., Navas, C.A., James, R.S., 2003. Tradeoffs and the evolution of thermal reaction norms. Trends Ecol. Evol. 18, 234–240.
Barlow, G.W., 1961. Intra-and interspecific differences in rate of oxygen consumption in gobiid fishes of the genus Gillichthys. Biol. Bull. 121, 209–229.
Barrionuevo, W.R., Fernandes, M.N., 1998. Time-course of respiratory metabolic adjustments of a south American fish, Prochilodus scrofa, exposed to low and high temperatures. J. Appl. Ichthyol. 14, 37–41.
Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using lme4. In: arXiv, (Preprint arXiv:1406.5823).
Bograd, S.J., Lynn, R.J., 2003. Long-term variability in the southern California Current System. Deep-Sea Res. II Top. Stud. Oceanogr. 50, 2355–2370.
Bograd, S.J., Chereskin, T.K., Roemmich, D., 2001. Transport of mass, heat, salt, and nutrients in the southern California Current System: annual cycle and interannual variability. J. Geophys. Res. 106, 9255–9275.
Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C., Vasseur, D.A., 2011. Why intraspecific trait variation matters in community ecology. Trends Ecol. Evol. 26, 183–192.
Bustamante, R.H., Branch, G.M., Eekhout, S., Robertson, B., Zoutendyk, P., Schleyer, M., Dye, A., Hanekom, N., Keats, D., Jurd, M., 1995. Gradients of intertidal primary productivity around the coast of South Africa and their relationships with consumer biomass. Oecologia 102, 189–201.
Cai, W., Borlace, S., Lengaigne, M., Van Rensch, P., Collins, M., Vecchi, G., Timmermann, A., Santoso, A., McPhaden, M.J., Wu, L., 2014. Increasing frequency of extreme El Niño events due to greenhouse warming. Nat. Clim. Chang. 4, 111.
Careau, V., Gifford, M.E., Biro, P.A., 2014. Individual (co) variation in thermal reaction norms of standard and maximal metabolic rates in wild-caught slimy salamanders. Funct. Ecol. 28, 1175–1186.
Cetina-Heredia, P., Roughan, M., Van Sebille, E., Feng, M., Coleman, M.A., 2015. Strengthened currents override the effect of warming on lobster larval dispersal and survival. Glob. Chang. Biol. 21, 4377–4386.
Clark, T.D., Sandblom, E., Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J. Exp. Biol. 216, 2771–2782.
Clarke, A., Johnston, N.M., 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. J. Anim. Ecol. 68, 893–905.
Donelson, J.M., Munday, P.L., 2012. Thermal sensitivity does not determine acclimation capacity for a tropical reef fish. J. Anim. Ecol. 81, 1126–1131.
Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale, M.K., Patterson, D.A., Hinch, S.G., Farrell, A.P., 2011. Differences in thermal tolerance among sockeye salmon populations. Science 332, 109–112.
Fangue, N.A., Hofmeister, M., Schulte, P.M., 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, Fundulus heteroclitus. J. Exp. Biol. 209, 2859–2872.
Faria, C., Almada, V.C., 2006. Patterns of spatial distribution and behaviour of fish on a rocky intertidal platform at high tide. Mar. Ecol. Prog. Ser. 316, 155–164.
Feary, D.A., Clements, K.D., 2006. Habitat use by triplefin species (Tripterygiidae) on rocky reefs in New Zealand. J. Fish Biol. 69, 1031–1046.
Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D., Hales, B., 2008. Evidence for upwelling of corrosive" acidified" water onto the continental shelf. Science 320, 1490–1492.
Futuyma, D.J., Moreno, G., 1988. The evolution of ecological specialization. Annu. Rev. Ecol. Syst. 19, 207–233.
Grosberg, R., Cunningham, C.W., 2001. Genetic structure in the sea. In: Marine Community Ecology. Sinauer, Sunderland, MA, pp. 61–84.
Guderley, H., Blier, P., 1988. Thermal acclimation in fish: conservative and labile properties of swimming muscle. Can. J. Zool. 66, 1105–1115.
Han, Y.-S., Lin, Y.-F., Wu, C.-R., Iizuka, Y., Castillo, T.R., Yambot, I.U., Mamalangkap, M.D., Yambot, A.V., 2016. Biogeographic distribution of the eel Anguilla luzonensis: dependence upon larval duration and oceanic currents. Mar. Ecol. Prog. Ser. 551, 227–238.
Harley, C.D., Randall Hughes, A., Hultgren, K.M., Miner, B.G., Sorte, C.J., Thornber, C.S., Rodriguez, L.F., Tomanek, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems. Ecol. Lett. 9, 228–241.
Hazel, J.R., Prosser, C.L., 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev. 54, 620–677.
Hickey, B.M., Dobbins, E.L., Allen, S.E., 2003. Local and remote forcing of currents and temperature in the central Southern California Bight. J. Geophys. Res. 108.
Kassen, R., 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. J. Evol. Biol. 15, 173–190.
Kawecki, T.J., Ebert, D., 2004. Conceptual issues in local adaptation. Ecol. Lett. 7, 1225–1241.
Kuo, E.S.L., Sanford, E., 2009. Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. Mar. Ecol. Prog. Ser. 388, 137–146.
Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M.J., Hinch, S.G., Healey, M.C., 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (Oncorhynchus nerka) and coho (O. kisutch) salmon stocks. J. Exp. Biol. 206, 3239–3251.
Legaard, K.R., Thomas, A.C., 2006. Spatial patterns in seasonal and interannual variability of chlorophyll and sea surface temperature in the California current. J. Geophys. Res. 111.
Lenormand, T., 2002. Gene flow and the limits to natural selection. Trends Ecol. Evol. 17, 183–189.
Leong, W., Sun, P.Y., Edmands, S., 2017. Latitudinal clines in temperature and salinity tolerance in tidepool copepods. J. Hered. 109, 71–77.
Lessios, H.A., Kessing, B.D., Robertson, D.R., 1998. Massive gene flow across the world's most potent marine biogeographic barrier. Proc. R. Soc. Lond. B Biol. Sci. 265, 583–588.
Love, M.S., 2011. Certainly More than you Want to Know about the Fishes of the Pacific Coast: A Postmodern Experience. Really Big Press.
Mayr, M., Berger, A., 1992. Territoriality and microhabitat selection in two intertidal New Zealand fish. J. Fish Biol. 40, 243–256.
Morris, R.W., 1961. Distribution and temperature sensitivity of some eastern Pacific cottid fishes. Physiol. Zool. 34, 217–227.
Norin, T., Malte, H., 2012. Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brown trout. Physiol. Biochem. Zool. 85, 645–656.
Norin, T., Malte, H., Clark, T.D., 2016. Differential plasticity of metabolic rate phenotypes in a tropical fish facing environmental change. Funct. Ecol. 30, 369–378.
Parrish, R.H., Nelson, C.S., Bakun, A., 1981. Transport mechanisms and reproductive success of fishes in the California current. Biol. Oceanogr. 1, 175–203.
Pfister, C.A., 1996. The role and importance of recruitment variability to a guild of tide pool fishes. Ecology 1928–1941.
Pfister, C.A., 1997. Demographic consequences of within-year variation in recruitment. Mar. Ecol. Prog. Ser. 153, 229–238.
Pfister, C.A., 2006. Concordance between short-term experiments and long-term censuses in tide pool fishes. Ecology 87, 2905–2914.
Poulin, R., Mouritsen, K.N., 2006. Climate change, parasitism and the structure of intertidal ecosystems. J. Helminthol. 80, 183–191.
Rangel, R.E., Johnson, D.W., 2018. Metabolic responses to temperature in a sedentary reef fish, the bluebanded goby (Lythrypnus dalli, Gilbert). J. Exp. Mar. Biol. Ecol. 501, 83–89.
Ritter, A.F., 2008. Habitat variation influences movement rates and population structure of an intertidal fish. Oecologia 157, 429–439.
Ritter, A.F., 2009. Regional variation in oceanographic conditions influences intertidal fish assemblage structure. Limnol. Oceanogr. 54, 1559.
Ritter, A.F., Preisler, R.K., 2006. Spatial variation in structure of an intertidal fish assemblage reflects daily settlement patterns. Mar. Ecol. Prog. Ser. 317, 211.
Sanford, E., Kelly, M.W., 2011. Local adaptation in marine invertebrates. Annu. Rev. Mar. Sci. 3, 509–535.
Schluter, D., 2000. The Ecology of Adaptive Radiation. OUP Oxford.
Schmidt-Nielsen, K., 1997. Animal Physiology: Adaptation and Environment. Cambridge University Press.
Shanks, A.L., 2009. Pelagic larval duration and dispersal distance revisited. Biol. Bull. 216, 373–385.
Shanks, A.L., McCulloch, A., Miller, J., 2003. Topographically generated fronts, very nearshore oceanography and the distribution of larval invertebrates and holoplankters. J. Plankton Res. 25, 1251–1277.
Slatkin, M., 1987. Gene flow and the geographic structure of natural populations. Science 236, 787–792.
Somero, G.N., 2005. Linking biogeography to physiology: evolutionary and acclimatory adjustments of thermal limits. Front. Zool. 2, 1.
Sorte, C.J.B., Jones, S.J., Miller, L.P., 2011. Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. J. Exp. Mar. Biol. Ecol. 400, 209–217.
Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. Science 301, 65.
Wang, G., Cai, W., Gan, B., Wu, L., Santoso, A., Lin, X., Chen, Z., McPhaden, M.J., 2017. Continued increase of extreme El Niño frequency long after 1.5° C warming stabilization. Nat. Clim. Chang. 7, 568.
Waples, R.S., 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41, 385–400.
Warner, R.R., Cowen, R.K., 2002. Local retention of production in marine populations: evidence, mechanisms, and consequences. Bull. Mar. Sci. 70, 245–249.
Wright, D., Bishop, J.M., Matthee, C.A., von der Heyden, S., 2015. Genetic isolation by distance reveals restricted dispersal across a range of life histories: implications for biodiversity conservation planning across highly variable marine environments. Divers. Distrib. 21, 698–710.
Xie, H., Lü, X., Zhou, J., Shi, C.C., Li, Y.L., Duan, T., Li, G., Luo, Y.P., 2017. Effects of acute temperature change and temperature acclimation on the respiratory metabolism of the snakehead. Turk. J. Fish. Aquat. Sci. 17, 535–542.
Yoshiyama, R.M., Sassaman, C., 1987. Geographical patterns of allozymic variation in three species of intertidal sculpins. Environ. Biol. Fish 20, 203–218.
Zaba, K.D., Rudnick, D.L., 2016. The 2014–2015 warming anomaly in the Southern California Current System observed by underwater gliders. Geophys. Res. Lett. 43, 1241–1248.
Zeng, L.-Q., Zhang, Y.-G., Fu, S.-J., Cao, Z.-D., 2011. Effects of acute temperature change on resting oxygen consumption rate and critical swimming speed in juvenile southern catfish (Silurus meridionalis Chen). Acta Hydrobiol. Sinica 35, 276–282.